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A STUDY OF THE PROPERTIES OF
PATHOGENIC STAPHYLOCOCCI
OF VARIOUS ORIGINS WHICH PRODUCE - TOXIN

COUNTRY: USSR

TECHNICAL TRANSLATION

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by

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13. ABSTRACT The distribution of δ -toxin producers has been studied among 175 pathogenic staphylococcus strains, 86% produced δ -toxin (δ -toxin alone in 12%, and in combination with α -toxin in 74%). δ -Toxin production was correlated with signs of staphylococcus pathogenicity. Strains producing δ -toxin were somewhat more resistant to antibiotics, but possessed lower dermonecrotic activity than α -toxin producers. Hemolytic titers of δ -toxin were established in relation to human erythrocytes, and its ability to lyse rabbit, sheep, horse, guinea pig, and monkey erythrocytes was demonstrated. 1 ←			

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A STUDY OF THE PROPERTIES OF
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Soviet and foreign investigators have introduced many innovations in the study of microbiology and immunology of staphylococcus infection (Burnet, 1929, 1930; Blair, 1958; Chistovich, 1961; Zygodchikov, 1963, etc.). As a result of this research the role of staphylococcal alpha-toxin in the pathogenesis of staphylococcus infection has been demonstrated and its basic properties and natures studied. A harmless derivative of alpha-toxin - staphylococcus anatoxin - is widely used for active immunization in total prophylaxis of staphylococcus infections.

The role and importance of other staphylococcus exotoxins in the pathogenesis of diseases of staphylococcal etiology and the feasibility of using them for immunoprophylaxis has still not been studied adequately. The most interesting in this regard are leucodysin, discovered in 1932 by Panton and Valentine, and delta-toxin reported in 1947 (Williams and Harper). In order to reveal the role and importance of delta-toxin in the pathogenesis of staphylococcus infections and its prospect for practical use, it is necessary to know the extent of distribution of delta-toxin producers among the pathogenic strains of staphylococcus of various origins. Williams and Harper (1947), Elek and Levy (1950, 1954), Marks and Vaughan (1950) in their research noted a correlation between producers of alpha- and delta-toxins, a wide distribution of strain-producers of delta-toxin among pathogenic strains of staphylococcus and a higher content of delta-toxin in strains isolated from carriers. In coagulase-negative strains delta-toxin was detected infrequently or not at all.

In later works (Adamczyk and Blaurock, 1963; Haque and Baldwin, 1964) presented statistical data on the distribution

of producers of one delta-toxin (15%) and alpha-, delta-toxins (60-70%) among staphylococcus strains isolated in abscesses and superficial festering processes. Of the Soviet works one may name the research of Konova (1965), which indicates the distribution of producers of alpha-, delta-, and beta-hemolysins among pathogenic staphylococcus strains isolated during food poisoning. Characteristics of staphylococcus delta-toxin are presented in monographs of Zygodchikov (1963) and Chistovich (1961).

On the basis of published data one may conclude that the breadth of distribution of delta-toxin producers and the biological properties of these strains has almost not been studied.

We have undertaken to reveal the presence and distribution of delta-toxin producers among pathogenic staphylococcus strains of various origins, to determine hemolytic activity of delta-toxin and to establish the connection between the presence of delta-toxin production and signs of pathogenicity of staphylococcus.

One hundred seventy-five strains of pathogenic staphylococcus were studied, isolated in 1965-1966 from healthy people and from pathological material from patients. Identification of the strains was accomplished according to the following symptoms: presence of pigment, gram staining, coagulation time of rabbit citrate plasma, phage-type, sensitivity to antibiotics (penicillin, streptomycin, biomycin, terramycin, levomycetin), dermonecrotic activity, lecithinase production (Zygodchikov, 1963). For determination of type of hemolysis, erythrocytes of various species of animals were used, seeding was carried out in 5% blood agar with whole blood, and in addition immunodiffusion was used according to the method of Elek and Levy (1950) with standard anti-alpha-serum, and the hemolytic titers of alpha- and delta-toxins were determined.

In studying the isolated strains of pathogenic staphylococci particular attention was paid to revealing producers of delta-toxin and determining its activity. In these strains parallel determinations were made of alpha-toxin titer (Table 1).

Table 1

A Source of isolated strains	Число штаммов	Продукция токсинов			D Распределение штаммов по титрам токсинов											
					α				δ				αδ			
					E		E		E		E		E		E	
					α	δ	α	δ	α	δ	α	δ	α	δ	α	δ
		α	δ	αδ	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
F Гной от больных маститом	36	1	3	32	—	1	—	—	2	1	15	9	8	3	22	7
G Фурункулез, пиодермия	40	7	3	20	—	6	1	1	2	—	1	14	15	8	13	9
H Слизь из полости носа и зева здоровых детей	42	5	5	32	—	4	1	1	3	1	9	8	15	6	20	6
I Слизь из полости носа и зева больных детей	17	2	6	9	1	1	—	1	4	1	—	4	5	1	2	6
J Послеоперационное нагноение	22	8	3	11	4	3	1	2	1	—	2	4	5	5	6	—
K Энтерит, энтероколит	8	—	—	8	—	—	—	—	—	—	1	3	4	—	2	6
L Всего . . . абс. M	165	23	20	122	5	15	3	5	12	3	28	42	52	23	65	34
	—	14	12	74	—	—	—	—	—	—	—	—	—	—	—	—

A. Source of isolated strains

B. Number of toxins

C. Toxin producers

D. Distribution of strains according to toxin titres

E. And above

F. Pus from mastitis patients

G. Furunculosis, pyoderma

H. Mucus from nasal and mouth cavities of healthy children

I. Mucus from nasal and mouth cavities of sick children

J. Postoperative suppuration

K. Enteritis, enterocolitis

L. Total

M. Abs.

On the basis of the data obtained one may make conclusions on the extent of distribution among pathogenic staphylococcus strains which produce delta-toxin; in this process the most frequently encountered strains were those producing alpha- and delta-toxins (74%), and strains producing only delta-toxins were encountered in 12% of the cases. A marked dependence of delta-toxin activity on the source of isolation was not noted, although among strains isolated during mastitis in women, a high percent of strains producing delta-toxins on the average also at high titers (91%) were revealed.

Table 2

А Продукция токсинов	В Число штам- мов	С Коагулаза		D Лецитиназа		Е Ферментирование							
						F Ферментация					G типирование д-с	H не типиро- вано	
						+	-	I	II	III			IV
δ I	23	22	1	16	7	2	2	13	1	—	18	5	
α I	22	22	0	11	11	7	1	6	—	5	19	3	
δ и α	129	129	1	92	38	11	12	66	2	6	97	33	
Всего абс.	175	173	2	119	56	20	15	85	3	11	134	41	
% J	100	98,9	1,2	68	32	15	11	63	2	8	77	23	

A. Toxin produced

B. Number of strains

C. Coagulase

D. Lecithinase

E. Phage-typing

F. Phage-group

G. Typed

H. Not typed

I. And

J. Total abs.

After studying the connection between delta-toxin and such symptoms of pathogenicity of staphylococci as production of coagulase, lechithinase, association with phage-type (Table 2), it was established that strains producing delta-toxin (alone or in combination with alpha-toxin) were pathogenic, and produced coagulase and lechithinase in a significant percent of cases. Coagulase production was one of the most essential signs of pathogenicity in staphylococci; it was determined according to our data in 98.8% of cases.

Lechithinase was detected in 68-70% of the strains, and consequently, cannot serve as a determining sign in establishing the pathogenicity of staphylococci.

After assigning the strains studied to one or another phage-type, one may note that the highest proportion belonged to the strains of Group III (63%), strains of Group I consisted of only 15 per cent. In all experiments phages were used in a dose of 100 TD (test dilution).

The ability to cause hemolysis in dense media with erythrocytes of various species of animals was also studied in the strains (Table 3).

		Table 3							
A	Лизис эритроцитов разных животных и человека	B Число изученных штаммов	C Число продуцентов токсинов						
			δ		α		α и δ		
			D всего	E гемо-лиз	D всего	E гемо-лиз	D всего	E гемо-лиз	
G H I J K L	Кролики	160	23	23	27	27	110	110	
	Человека	165	25	25	27	2	104	104	
	Лошади	87	17	16	16		54	47	
	Бараны	87	15	15	15	10	56	50	
	Морской свинки	108	20	20	17	3	68	65	
	Обезьяны	43	12	12	5	1	24	24	

A. Lysis of erythrocytes of various animals and man.

B. Number of strains studied

C. Number of toxin-producers

D. Total

E. Hemolysis

F. And

G. Rabbit

H. Human

I. Horse

J. Sheep

K. Guinea pig

L. Monkey

The data obtained permitted conclusions on the breadth of the hemolytic spectrum of delta-toxin of staphylococcus, which lysed all of the species of erythrocytes studied, forming a zone of hemolysis 1-2 mm in width with clearly defined margins on blood agar. The most suitable proved to be erythrocytes from humans, horses and guinea pigs, whose application permitted differentiation of delta-toxin from other toxins. Alpha-toxin either did not lyse the erythrocytes of these

species, or weakly lysed them, depending on its strength.

Table 4

Таблица 4

A Продуцируе- мая токсин	B Число штаммов	C Чувствительность к антибиотикам					H левоме- цину
		D пеници- лину	E стрепто- мицину	F тетраци- цину	G биомици- ну		
δ	24	2	7	3	6	7	
α	22	2	11	8	9	10	
α и δ	138	18	42	42	45	52	
Всего abs.	184	22	60	53	60	69	
J %	100	14	33	32	33	38	

K * Из-за отсутствия тетрациклина в начале ра-
боты у некоторых штаммов чувствительность
к данному антибиотику не определяли.

- A. Toxin produced
- B. Number of strands
- C. Sensitivity to antibiotics
- D. Penicillin
- E. Streptomycin
- F. Terramycin
- G. Biomycin
- H. Levomycetin
- I. And
- J. Total abs.

K Because of the absence of terramycin at the be-
ginning of the work the sensitivity of certain
strains to the given antibiotic was not determined.

We have determined the sensitivity of the strains studied
to the five antibiotics most frequently required for treat-
ment - penicillin, streptomycin, biomycin, terramycin and
levomycin (Table 4).

The majority of strains was resistant to penicillin (86%),
the sensitivity of the strains to the remaining four antibio-
tics did not vary sharply. A somewhat high percent of resis-
tant strains was found among delta-producers.

A significant portion of the strains studied possessed
dermonecrotic activity (Table 5), while the majority of
strains caused necrosis at the minimal dose studied (100
million microbial cells).

One must note that the strains producing alpha-toxin (alone
or in combination with delta-toxin) possessed a marked dermo-
necrotic activity, and the delta-producers proved less active.

CONCLUSIONS

1. A wide distribution among pathogenic staphylococci

Table 5

A Продуцируемый токсин	B Число штаммов	C Частота реак- ции			E Отрицательная
		D Положи- тельной с разной ми- нимальной дермоне- ротической дозой (в млн. мик- робных клеток)			
		100	200	400	
α	16	9	2	5	0
δ	14	5	3	3	3
α и δ	71	46	14	7	4
Всего	101	60	19	15	7

G

- A. Toxin produced
- B. Number of strains
- C. Frequency of reaction
- D. Positive with various minimal der-
monecrotic dose (in millions of micro-
bial cells)
- E. Negative
- F. And
- G. Total

of strains producing delta-toxin has been established (864); moreover, the majority of strains produced delta-toxin with hemolytic titres from 1:20 to 1:80.

2. Delta-toxin possessed a broad hemolytic spectrum.

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